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Effect of Conditioning Regimen Intensity on CMV Infection in Allogeneic Hematopoietic Cell Transplantation

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Nonmyeloablative conditioning is less toxic and results in initial establishment of mixed hematopoietic T cell chimerism for up to half a year with prolonged presence of host T cell immunity. In this study, we examined whether this translates into differences in the risks and/or severity of cytomegalovirus (CMV) infection and disease. We analyzed data from 537 nonmyeloablative (NM-HCT) and contemporaneous 2489 myeloablative hematopoietic cell transplant (M-HCT) recipients. In CMV seropositive recipients, no difference in the overall hazards of CMV infection at any level (adjusted hazard ratio [adj. HR] 0.9, 95% confidence interval [95% CI]: 0.7-1.0, $P = .14$) was noted; however, NM-HCT was associated with a lower risk of high-grade CMV infection (adj. HR 0.7, 95% CI: 0.5-0.9, $P = .02$). CMV disease rates were similar between the groups during the first 100 days after HCT, but NM-HCT recipients had an increased risk of late CMV disease (adj. HR 2.0, 95% CI 1.2-3.4). The increased risk of late CMV disease after NM-HCT was pronounced during the earlier years of the study period, but not detectable in more recent years. Contrary to earlier reports, survival following CMV disease was not reduced after NM-HCT when compared to M-HCT recipients. These results suggest that residual host cells after NM-HCT reduce progression to higher CMV viral load in NM-HCT recipients; however, this effect does not appear to protect against serious complications of CMV. Therefore, CMV prevention strategies in NM-HCT recipients should be similar to those used in M-HCT recipients.

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INTRODUCTION

Nonmyeloablative HCT (NM-HCT) is now widely used in patients with hematologic and nonhematologic malignancies who are ineligible for myeloablative HCT (M-HCT) because of advanced age or comorbidities [1-3]. NM-HCT includes reduced or minimally intensive conditioning therapy before transplantation, combined with more intensive immunosuppression after transplantation. The nonmyeloablative regimen used in Seattle consists of low-dose total body irradiation (TBI) 2 Gy with or without fludarabine (Flu) followed by cyclosporine (CsA) or tacrolimus and mycophenolate mofetil (MMF) as postgrafting immunosuppression [3-9]. This regimen causes minimal toxicity, and results in initial establishment of mixed host/donor T cell chimerism for up to approximately 6 months. Thus, risk or severity of viral infections, such as cytomegalovirus (CMV) infection, may be reduced.

We initially reported that the time of CMV disease onset was delayed after HLA matched-related NM-HCT, supporting the hypothesis that extended presence of host memory immune responses after NM-HCT might play a role in protection against early CMV infection [10-12]. We also reported that the risk of CMV disease was similar after NM-HCT from HLA matched-unrelated and -related donors [13]. Our study also suggested that CMV disease might be associated with a lower mortality in NM-HCT

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recipients [10]. These studies were done early after the technique was introduced, which limited the power of statistical analyses because of small sample sizes.

This report examines the incidence, risk, and outcome of CMV infection in a large cohort of recipients undergoing NM-HCT and compared the outcomes with those of contemporaneous M-HCT recipients.

PATIENTS AND METHODS

This retrospective analysis was approved by the institutional review board of the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, WA). Informed consent was provided according to the Declaration of Helsinki.

Patients

We compared outcomes in 537 consecutive patients who received NM-HCT between December 1997 and December 2005, at the FHCRC and 2489 patients who received M-HCT between January 1995 and December 2005, at the FHCRC (Table 1). Recipient and donor age, proportions of a prior transplant history, peripheral stem cell source, CMV high-risk patients, and MMF use as a postgrafting immune suppressant were significantly higher in NM-HCT compared to M-HCT. NM-HCT became more common in the later years, and no T cell-depletion regimen was used in NM-HCT.

The CMV risk was stratified into 3 groups: low (recipient negative and donor negative); intermediate (recipient negative and donor positive); and high (recipient positive and either donor negative or positive) based on recipient and donor CMV serostatus before HCT.

Preparative Conditioning Regimens and Sources for HCT

Three hundred forty-eight (64.8%) NM-HCT recipients received fludarabine (Flu; 30 mg/m^2/day for 3 consecutive days) and low-dose TBI (2 Gy, day 0), whereas 90 patients (16.8%) received low-dose TBI (2 Gy, day 0) alone. Patients in the M-HCT group received different types of conditioning. The most common regimens consisted of cyclophosphamide (Cy; 60 mg/kg/day for 2 consecutive days) followed by TBI (12-13.2 Gy) or busulfan (Bu; 4 mg/kg/day for 4 consecutive days) followed by Cy (60 mg/kg/day for 2 consecutive days) in 805 (32.3%) and 779 (31.3%) patients, respectively (data not shown). The distribution of stem cell sources used in NM-HCT and M-HCT is shown in Table 1.

Prophylaxis and Diagnosis of Graft-versus-Host Disease (GVHD)

GVHD prophylaxis regimens are shown in Table 1. NM-HCT patients most commonly received CsA and MMF orally as immune suppressants post HCT. MMF was administered 15 mg/kg orally twice a day from day 0 to day 27 and discontinued for the HLA matched-related NM-HCTs and MMF 15 mg/kg orally 2 or 3 times a day from day 0 to day 40, with a taper to day 96 for the unrelated NM-HCTs. For the single HLA-antigen and combined HLA-antigen and allele mismatched NM-HCTs, 15 mg/kg MMF was given 3 times a day and was tapered at day 100 over 2 months [5-9].

M-HCT patients most commonly received the combination of CsA and MTX. CsA was given at a dose of 1.5 mg/kg intravenously (i.v.) twice a day or 6.25 mg/kg orally twice a day. MTX was administered i.v. at a dose of 15 mg/m^2 on day 1, and 10 mg/m^2 on day 3, 6, and 11. Diagnoses of acute or chronic GVHD (aGVHD, cGHVD) were performed according to established criteria [14,15].

Infection Surveillance and Preemptive Therapy against CMV

CMV surveillance including polymerase chain reaction (PCR), pp65 antigenemia (AG), and blood culture was performed on a weekly basis until day 100. After day 100, surveillance and preemptive therapy were recommended for CMV intermediate and high-risk patients on a weekly or biweekly basis. Patients were monitored for the development of CMV infection and diseases until day 365. CMV pp65 AG was quantified as the average number of positive cells per 200,000 peripheral blood leukocytes and quantitative detection of CMV DNA in plasma by PCR was performed as described [16].

Ganciclovir (GCV) treatment was started when CMV AG/PCR became positive during the first 100 days after HCT. All patients with CMV AG at any level received GCV (5 mg/kg i.v. twice daily) for 7 to 14 days as induction therapy, followed by maintenance therapy with a half dose of GCV (5 mg/kg i.v. daily) or valganciclovir 900 mg once a day orally until negative surveillance testing was detected or day 100. After day 100, preemptive therapy consisting of i.v. GCV or valganciclovir induction, followed by maintenance therapy, was recommended when CMV AG became positive or when PCR was >1000 copies/mL. GCV was substituted with foscarnet in patients with neutropenia.

Between January 1995 and November 1998, no patients received acyclovir for varicella zoster virus (VZV) prevention; but herpes simplex virus (HSV)-positive recipients were given acyclovir, 250 mg/m^2 twice daily from day −7 until engraftment and resolution of mucositis. From November 1998 until May 2002, VZV seropositive HCT recipients received prophylaxis against VZV (acyclovir 250 mg/m^2 i.v., followed by 800 mg orally or valacyclovir 500 mg orally; all drugs given twice per day for 1 year after

Table 1. Characteristics of the Study Cohort

Variable	M-HCT (n = 2489) Number (%)	NM-HCT (n = 537) Number (%)	*P*-Value
Median (range) age, years			
Patient	39.8 (0.5-67.0)	54.2 (0.5-74.5)	<.0001
Donor	38.2 (0.7-81.7)	42.5 (1.3-83.3)	<.0001
Sex			
Male	1422 (57.1)	334 (62.2)	
Female	1067 (42.9)	203 (38.6)	.03
Donor sex			
Male	1399 (56.2)	284 (52.9)	
Female	1087 (43.7)	253 (47.1)	.26
Recipient race			
Caucasian	2015 (81.0)	470 (87.5)	
Other/unknown	474 (19.0)	67 (12.5)	<.001
Donor race			
Caucasian	1457 (58.5)	269 (50.1)	
Other/unknown	1032 (41.5)	268 (49.9)	<.001
Year of transplantation			
1995-1997	834 (33.5)	1 (0.2)	
1998-2000	716 (28.8)	116 (21.6)	
2001-2003	578 (23.2)	225 (41.9)	
2004-2005	361 (14.5)	195 (36.3)	<.0001
Prior transplant (auto and/or allo)			
Yes	74 (3.0)	202 (37.6)	
No	2415 (97.0)	335 (62.4)	<.0001
Source of stem cell			
BM	1431 (57.5)	49 (9.1)	
PBSC	1015 (40.8)	487 (90.7)	
Cord	43 (1.7)	1 (0.2)	<.0001
HLA matching			
Matched related	1017 (42.0)	221 (44.0)	
Mismatched related/unrelated	1404 (56.4)	281 (52.3)	.28
CMV seropositive			
Yes	1260 (50.6)	311 (57.9)	
No	1228 (49.3)	225 (41.9)	<.01
Donor CMV seropositive			
Yes	984 (39.5)	230 (42.8)	
No	1503 (60.4)	307 (57.2)	.30
CMV risk			
Low	894 (35.9)	152 (28.3)	
Intermediate	334 (13.4)	73 (13.6)	
High	1260 (50.6)	311 (57.9)	<.01
Disease diagnosis			
AA	67 (2.7)	2 (0.4)	
ALL	346 (13.9)	21 (3.9)	
AML	702 (28.2)	143 (26.6)	
CLL	18 (0.7)	40 (7.5)	
CML	669 (26.9)	18 (3.4)	
HL	14 (0.6)	46 (8.6)	
MDS	465 (18.7)	41 (7.6)	
MM	38 (1.5)	83 (15.5)	
NHL	106 (4.3)	98 (18.3)	
Congenital disorders	19 (0.8)	16 (3.0)	
Other	44 (1.8)	29 (5.4)	<.0001
T cell depletion regimen			
Yes	270 (10.9)	0 (0.0)	
No	2219 (89.2)	537 (100.0)	<.0001
Acute GVHD prophylaxis			
Calcineurin inhibitor only	27 (1.1)	17 (3.2)	
Calcineurin inhibitor +MMF	66 (2.7)	495 (92.2)	
Calcineurin inhibitor +MTX	2294 (92.2)	5 (0.9)	
Other	101 (4.1)	20 (3.7)	<.0001

AA indicates aplastic anemia; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; HL, Hodgkin lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; CMV, cytomegalovirus; BM, bone marrow; PBSC, peripheral blood stem cell; acute GVHD, acute graft-versus-host disease; MMF, mycophenolate mofetil; MTX, methotrexate.

T cell depletion regimens are those containing antithymocyte globulin or anti-CD3 antibody, BC3.

P-values from chi-square test, Fisher's exact test, or Wilcoxon rank-sum test as appropriate.

transplantation (valacyclovir was preferred for patients who received >0.5 mg/kg per day of steroids). HCT patients undergoing transplantation after May 2002 received the same regimen until 1 year after transplantation. In patients who were still receiving inmmunosuppression at 1 year, acyclovir/valacyclovir prophylaxis was continued until 6 months after discontinuation of all immunosuppression [17].

Definitions of CMV Infection and Disease

CMV AG was diagnosed by blood pp65 antigen testing, CMV viremia by positive blood culture or shell vial centrifugation culture [10], and detection of CMV DNA by PCR [16]. CMV disease was defined by established criteria [18].

Study Endpoints

In the present study, we evaluated the following 6 endpoints: (1) CMV infection (any CMV AG/DNA detection) by day 100; (2) high-grade CMV infection (CMV AG >10/200,000 peripheral blood leukocytes or PCR >1000 copies/mL by day 100; (3) CMV viremia (culture) by day 100; (4) CMV disease by day 100 and 1 year; (5) late CMV disease, which occurred after day 100 after HCT; (6) survival after CMV disease.

Statistical Analysis

Characteristics of NM-HCT and M-HCT patients were summarized using frequency counts and percentages for categoric variables and medians and ranges for continuous variables.

The cumulative incidences of CMV infection, CMV high-grade infection, CMV disease, and late CMV disease were compared between NM-HCT and M-HCT groups, with subsequent transplantation or death considered competing risks. Cumulative incidence curves for these endpoints also were stratified by CMV risk groups defined by donor and recipient seropositivity and by transplant year groupings between 1995 and 2005. The probability of survival was estimated by the Kaplan-Meier method. Hazards were compared using the log-rank test. The median times to onset of CMV disease were compared by the Wilcoxon rank sum test.

Univariate and multivariate Cox regression models were used to estimate hazard ratios and 95% confidence intervals (95% CI). Cox regression analyses for CMV infection, high-grade CMV infection, CMV viremia, and CMV disease were performed in the CMV high-risk group. CMV viremia was analyzed in just a slightly smaller subset because CMV viremia was tested only through 12/2003. Covariates included recipient/donor age and sex, recipient/donor race, donor CMV serostatus, sex mismatch, HLA disparity, donor relationship, intensity of conditioning, stem cell source, HSV type I, II serostatus, T cell-depleted conditioning, transplantation year, disease risk, GVHD prophylaxis, aGVHD, and cGVHD. Additionally, CMV risk group and maximum values of CMV AG and PCR testing were evaluated as risk factors for CMV disease.

aGVHD and cGVHD, CMV AG, and PCR testing were analyzed as time-dependent variables. Variables less than $P = .05$ in the univariate models were candidates for the multivariate models. Nonmyeloablative versus myeloablative conditioning was forced into multivariate models of all endpoints.

RESULTS

CMV Infection and Viremia

Low- and intermediate-risk group (D−/R−, D+/R−)

The cumulative incidences of CMV infection by day 100 were similar between NM-HCT and M-HCT in CMV low- and intermediate-risk groups. High-grade CMV infection was very rare, particularly in CMV low-risk groups and was not noted in any low-risk NM-HCT patient (Table 2).

High-risk group (D−/R+, D+/R+)

In CMV high-risk group (n = 1571), there were trends toward lower CMV infection and high-grade CMV infection rates in NM-HCT (Table 2 and Figure 1). When high viral load was analyzed, stratified by HLA-matched related versus unrelated/HLA-mismatched related donor status in the CMV high-risk group, the lower incidence of high-grade

Table 2. The Incidences of CMV Infection and Disease

Endpoints	Incidence in M-HCT	Incidence in NM-HCT	*P*-Value
CMV infection by day 100			
Low risk	0.03	0.02	.64
Intermediate risk	0.15	0.21	.27
High risk	0.66	0.62	.08
High-grade CMV infection by day 100			
Low risk	0.01	0.00	.33
Intermediate risk	0.05	0.09	.20
High risk	0.25	0.14	<.0001
CMV disease by day 100			
Low risk	0.01	0.01	.52
Intermediate risk	0.01	0.02	.68
High risk	0.10	0.07	.11
CMV disease by 1 year			
Low risk	0.01	0.01	.96
Intermediate risk	0.03	0.03	.95
High risk	0.15	0.15	.50
Late CMV disease			
Low risk	0.01	0.00	.35
Intermediate risk	0.02	0.04	.68
High risk	0.09	0.11	.52

CMV indicates cytomegalovirus; M-HCT, myeloablative hematopoietic cell transplantation; NM-HCT, nonmyeloablative hematopoietic cell transplantation.

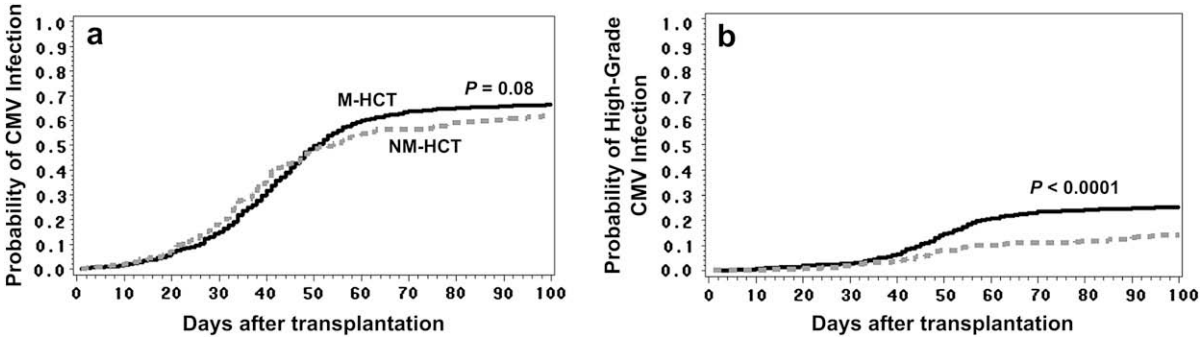

Figure 1. Cumulative incidences of any and high-grade CMV infections in CMV high-risk patients. The probabilities of (a) any CMV infection and (b) high-grade CMV infection (CMV AG >10 cells/200,000 PBL or CMV DNA >1000 copies/mL of plasma) in CMV high-risk patients are displayed. The dashed line indicates nonmyeloablative hematopoietic cell transplantion (NM-HCT) and the solid line indicates myeloablative hematopoietic cell transplantion (M-HCT). *P*-values were calculated by the log-rank test.

CMV infection in NM-HCT was seen both in the HLA-matched related and unrelated/HLA-mismatched related settings (Figure 2). Unrelated and HLA-mismatched donor status was associated with a somewhat higher cumulative incidence of high-grade CMV infection at day 100 than HLA-matched related donor status (Figure 2). This effect was seen in both M-HCT and NM-HCT recipients. In the CMV high-risk group, other factors associated with increased risks of any CMV infection were recipient age and aGVHD (III or IV). Other factors associated with an increased risk of high-grade CMV infection were recipient race (other than Caucasian) and aGVHD (Table 3).

Among seropositive patients transplanted between 1995 and 2003 (n = 1263), the incidence of CMV viremia (culture proven) by day 100 was significantly lower in NM-HCT compared with M-HCT (10% versus 19%, $P < .001$). However, in the multivariate model, the significance was not sustained. Other risk factors for CMV viremia were recipient race (other than Caucasian) (adj. hazard ratio [HR] 1.9, 95% CI: 1.2-3.0, $P < .01$) and aGVHD II to IV (adj. HR 3.4, 95% CI: 2.2-5.2, $P < .0001$). CMV viremia was less common in the later years of the study period (2001-2003) compared to 1995-1997 (adj. HR 0.4, 95% CI: 0.6-0.6, $P < .0001$) (data not shown).

Time to CMV Negativity after Start of Preemptive Therapy

As a surrogate marker for duration of anti-CMV treatment, we compared the duration from first positive to the first negative AG/PCR result between NM-HCT and M-HCT and found no difference in all patients (median [range]:7 [0-13] versus 7 [0-50] days, respectively, $P = .98$) or in patients at high risk for CMV (seropositive recipients) (median [range]:7 [0-13] versus 7 [0-50] days, respectively, $P = .97$).

CMV Disease and Survival after CMV Disease

Low- and intermediate-risk group (D−/R−, D+/R−)

Among CMV low- and intermediate-risk groups, there was no significant statistical difference in the

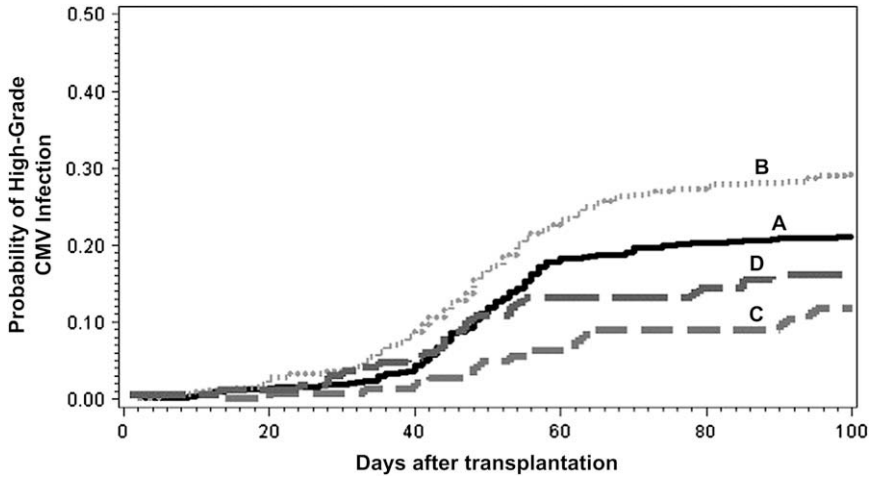

Figure 2. Cumulative incidences of CMV high-grade infection in CMV high-risk patients stratified by matched-related versus unrelated/HLA-mismatched donor. (A) myeloablative HLA-matched related donor, (B) myeloablative unrelated/HLA-mismatched related donor, (C) nonmyeloablative HLA-matched related donor, (D) nonmyeloablative unrelated/HLA-mismatched related donor.

Table 3. Univariate and Multivarite Analyses of Risk Factors for CMV Infection in CMV High-Risk Group

Factors	CMV Infection*				CMV High-Grade Infection*			
	Univariate HR (95%CI)	*P*	adj. HR (95%CI)	*P*	Univariate HR (95%CI)	*P*	adj. HR (95%CI)	*P*
NM-HCT	0.9 (0.7-1.0)	.08	0.9 (0.7-1.0)	.14	0.5 (0.4-0.7)	<.0001	0.7 (0.5-0.9)	.02
Recipient age ≥ 41 years	1.3 (1.1-1.4)	<.001	1.5 (1.3-1.7)	<.0001	0.9 (0.8-1.1)	.46	—	—
Recipient race (other than Caucasian)	1.2 (1.0-1.4)	.01	1.1 (0.9-1.4)	.23	1.2 (1.0-1.6)	.08	1.4 (1.1-1.9)	<.01
Donor CMV positive	1.0 (0.9-1.1)	.56	—	—	0.7 (0.6-0.9)	.002	0.7 (0.6-0.9)	<.01
Related donor	0.8 (0.7-0.9)	<.0001	0.9 (0.7-1.2)	.46	0.7 (0.5-0.8)	.0001	0.6 (0.4-1.0)	.04
HSV1 positive (recipient)	0.7 (0.5-0.8)	<.001	0.6 (0.5-0.8)	<.001	1.0 (0.7-1.6)	.92	—	—
Acute GVHD (III or IV)	1.5 (1.3-1.8)	<.0001	1.4 (1.2-1.6)	<.0001	2.2 (1.8-2.8)	<.0001	1.8 (1.4-2.3)	<.0001
Year of transplant								
1995-1997	1.0		1.0		1.0		1.0	
1998-2000	0.8 (0.7-1.0)	.01	0.8 (0.7-1.0)	.01	0.7 (0.5-0.9)	.006	0.7 (0.5-0.9)	<.01
2001-2003	0.8 (0.7-1.0)	.02	0.9 (0.7-1.1)	.20	0.5 (0.4-0.7)	<.0001	0.6 (0.4-0.8)	<.001
2004-2005	0.8 (0.7-1.0)	.04	0.8 (0.6-1.0)	.05	0.4 (0.3-0.5)	<.0001	0.4 (0.3-0.6)	<.0001

adj. HR indicates adjusted hazard ratio; CI, confidence interval; NM-HCT, nonmyeloablative hematopoietic cell transplantation; CMV, cytomegalovirus; HSV1, herpes simplex virus type 1; GVHD, graft-versus-host disease.
The factors that were statistically significant in either multivariate model of CMV infection or high-grade CMV infection, and NM-HCT are displayed.
*Total sample size of CMV high-risk patients is 1,571.

risk for CMV disease by day 100 and by 1 year between NM-HCT and M-HCT (Table 2).

High-risk (D−/R+, D+/R+)

In the CMV high-risk group, the cumulative incidence of CMV disease by day 100 (but not by 1 year) tended to be less frequent in NM-HCT compared with M-HCT (Tables 2 and 4). A significant decline of CMV disease incidence was noted after 2001 compared to 1995-2000 (Figure 3a and b). The cumulative incidence for CMV disease in CMV high-risk NM-and M-HCT patients from 1995 to 2000 were 27% versus 18% ($P = .25$) compared to 12% versus 11% ($P = .94$) between 2001 and 2005 (Figure 3a and b). A significant delay in the onset of CMV disease was observed in NM-HCT in the high-risk CMV group (median day of onset; 106.5 [6.0-1273.0] versus 69.5 days [6.0-1775.0] $P = .02$). Among NM-HCT, the cumulative incidences of CMV disease at 1 year between HLA-matched related

Table 4. Univariate and Multivariate Analyses of Risk Factors for CMV Disease in CMV High-Risk Group

Factors	CMV Disease*				Late CMV Disease*			
	Univariate HR (95%CI)	*P*	adj. HR (95%CI)	*P*	Univariate HR (95%CI)	*P*	adj. HR (95%CI)	*P*
NM-HCT	0.9 (0.7-1.3)	.74	1.3 (0.9-1.9)	.12	1.2 (0.7-1.8)	.52	2.0 (1.2-3.4)	.01
Donor sex (female)	1.3 (1.0-1.6)	.08	1.4 (1.1-1.7)	.02	1.2 (0.8-1.8)	.31	—	—
Unrelated/HLA-mismatched	1.4 (1.1-1.8)	<.01	1.2 (0.7-2.1)	.44	2.5 (1.7-3.9)	<.0001	2.1 (1.4-3.3)	<.01
HSV1 positive	2.0 (1.0-3.9)	.04	2.3 (1.2-4.5)	.01	2.6 (0.8-8.5)	.12	—	—
Acute GVHD (III or IV)	2.7 (2.1-3.6)	<.0001	2.1 (1.6-2.7)	<.0001	—	—	—	—
Chronic GVHD	2.6 (1.8-3.8)	<.0001	2.1 (1.4-3.0)	<.0001	—	—	—	—
Acute (III or IV) or Chronic GVHD	—	—	—	—	6.0 (2.6-13.6)	<.0001	4.1 (1.8-9.5)	<.01
Year of transplant								
1995-1997	1.0		1.0		1.0		1.0	
1998-2000	1.2 (0.9-1.7)	.24	1.3 (0.9-1.8)	.14	0.7 (0.4-1.8)	.14	0.7 (0.4-1.1)	.12
2001-2003	0.7 (0.5-0.9)	.02	0.8 (0.5-1.4)	.44	0.5 (0.3-0.8)	<.01	0.4 (0.2-0.8)	<.01
2004-2005	0.6 (0.4-0.9)	.02	0.8 (0.5-1.3)	.31	0.6 (0.3-1.0)	.05	0.5 (0.3-0.9)	.03
Max. CMV AG								
0	1.0		1.0		—		—	
>0-2/200,000	1.7 (1.1-2.5)	.01	1.7 (1.1-2.5)	.01	—	—	—	—
>2-10/200,000	1.6 (1.0-2.6)	.05	1.4 (0.9-2.3)	.16	—	—	—	—
>10/200,000	4.7 (3.3-6.8)	<.0001	3.7 (2.5-5.4)	<.0001	—	—	—	—
Max. CMV PCR (copies/mL)								
0	1.0		1.0		—		—	
0-1000	2.3 (1.4-3.8)	<.01	2.4 (1.4-4.1)	<.01	—	—	—	—
>1000	2.4 (1.2-4.6)	.01	3.2 (1.6-6.5)	<.01	—	—	—	—
Max. CMV AG>10/PCR >1000 before day 100 (copies/mL)	—	—	—	—	2.8 (1.9-4.1)	<.0001	2.0 (1.4-3.0)	<.01

adj. HR indicates adjusted hazard ratio; CI, confidence interval; NM-HCT, nonmyeloablative hematopoietic cell transplantation; CMV, cytomegalovirus; HSV1, herpes simplex virus type 1; GVHD, graft-versus-host disease; AG, antigenemia; PCR, polymerase chain reaction.
The factors that were statistically significant in either multivariate model of CMV disease or late CMV disease, and NM-HCT are displayed
*The sample size of CMV high-risk patients at risk for CMV disease is 1,571; the sample size of CMV high-risk patients who survived CMV-disease-free to day 100 and were at risk for late CMV disease is 1,109.

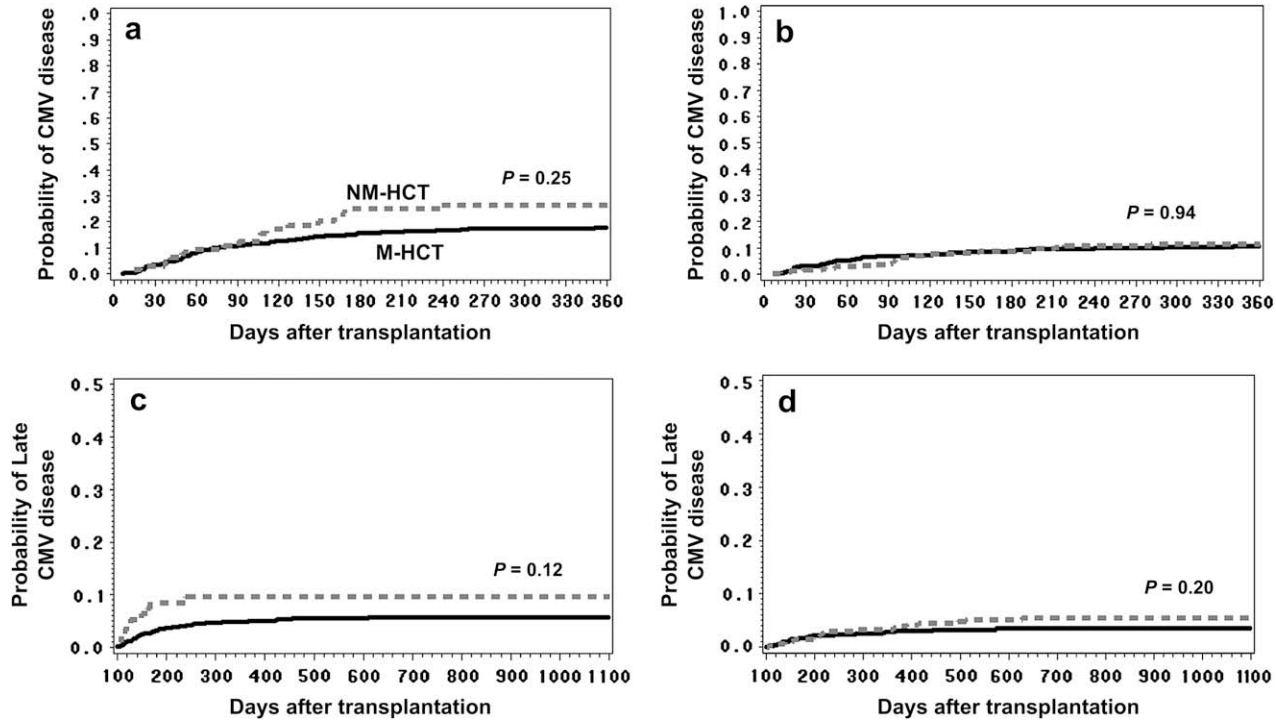

Figure 3. Cumulative incidences of CMV disease in CMV high-risk patients and late CMV disease. The probabilities of (a) CMV disease from1995 to 2000, (b) CMV disease from 2001 to 2005 in CMV high-risk patients, (c) late CMV disease from1995 to 2000, and (d) late CMV disease from 2000 to 2005 are displayed. The probability curves for late CMV were generated from all CMV high-risk patients who survived beyond day 100 without underlying disease relapse. The dashed line indicates nonmyeloablative hematopoietic cell transplantion (NM-HCT) and the solid line indicates myeloablative hematopoietic cell transplantion (M-HCT). *P*-values were calculated by the log-rank test.

and unrelated/HLA-mismatched related donors were very similar (15% versus 14%, *P*= .87).

Other risk factors associated with CMV disease in multivariate analysis were donor female sex, aGVHD, cGVHD, HSV type I seropositivity, and positivity of CMV AG and/or PCR (Table 4).

The probability of survival in CMV high-risk patients who developed CMV disease (n = 226) was not significantly different between NM-HCT and M-HCT recipients (*P* = .88) (Figure 4).

Late CMV Disease

Low- and intermediate-risk group (D−/R−, D+/R−)

No significant statistically differences in incidences were observed between MN-HCT and M-HCT. Late

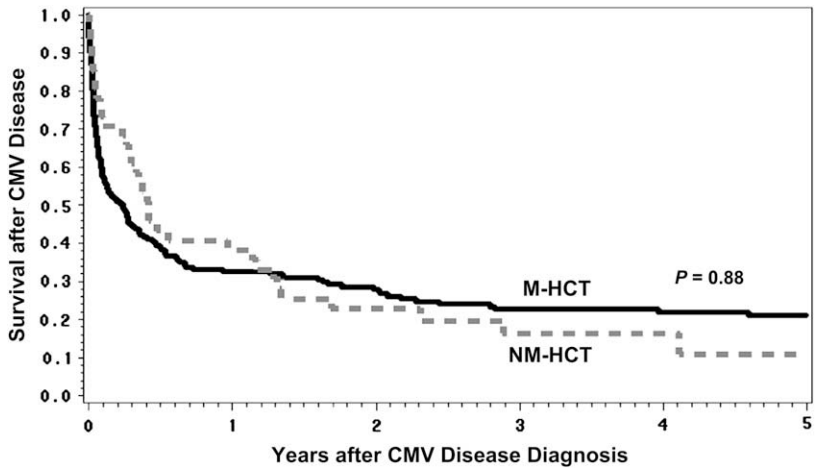

Figure 4. Survival after CMV disease in CMV high-risk patients. The probability of survival after CMV disease in 226 high-risk patients who had CMV disease. The dashed line indicates nonmyeloablative hematopoietic cell transplantion (NM-HCT) and the solid line indicates myeloablative hematopoietic cell transplantion (M-HCT). *P*-values were calculated by the log-rank test.

CMV disease was not observed in any CMV low-risk NM-HCT patient (Table 2).

High-risk group (D−/R+, D+/R+)

No statistically significant differences in late CMV disease incidences were detected between MN-HCT and M-HCT in CMV high-risk group (Table 2).

Among all the risk groups, NM-HCT was significantly associated with late CMV disease after adjustment for multiple covariates (adj. HR 2.0, $P = .01$) (Table 4). However, this was mainly driven by a high incidence of late CMV disease during the earlier years of the study period (Figure 3c and d). Additional risk factors for late CMV disease were: HLA-mismatch or unrelated donor, aGVHD (III or IV) or cGVHD before day 100, and maximum CMV AG >10/PCR > 1000 copies/mL before day 100. Furthermore, late CMV disease was less common in more recent years (2001-2005) compared to 1995-1997 (Table 4).

Secondary Invasive Bacterial and Fungal Infection after CMV Infection

We compared the incidences of secondary bacterial infection before day 100 and fungal infections before 1 year after HCT between NM-HCT and M-HCT. There was no significant difference in risk of probable and definite invasive fungal infection between NM-HCT and M-HCT in all CMV risk groups ($P = .77$), nor in high-CMV-risk group ($P = .83$).

Secondary invasive bacterial infections were less common in NM-HCT (23% versus 28%, chi-square value $P < .0001$). There was a significant difference in hazard of bacterial infection between NM-HCT and M-HCT adjusted for CMV risk group (HR = 0.6, 95% CI = 0.5-0.8, $P < .0001$); when the analysis was restricted to the CMV high-risk group (seropositive recipients), a similar effect was seen (HR = 0.7, 95% CI = 0.5-0.9, $P < .01$).

DISCUSSION

We comprehensively examined risks and outcomes of CMV infection and disease in a large cohort of uniformly treated patients that provided the necessary power to analyze CMV endpoints in NM-HCT recipients. NM-HCT recipients had similar rates of CMV infection and disease compared to M-HCT, although a delayed timing of disease and lower maximum CMV viral loads were noted. Contrary to an earlier small study that showed a trend toward improved outcome of CMV disease in NM-HCT [10], the present study did not show evidence of such an effect.

In a previous study, we demonstrated that NM-HCT showed trends toward lower incidence of CMV infection pp65 antigenemia, CMV viremia, and CMV disease during the first 100 days after HCT. However, we did not show statistically significant differences in the incidence of these CMV events between NM-HCT and M-HCT, possibly because of the small sample size [10]. In the present study, we were able to provide statistical evidence that the incidence of high CMV viral load in NM-HCT is lower compared with M-HCT. This effect was seen in both HLA mismatched-related or unrelated and HLA matched-related HCT recipients (Figure 2). Similar to an earlier study, there was a trend toward a more profound reduction of high CMV load in HLA-matched-related NM-HCT recipients than in HLA-mismatched related or unrelated NM-HCT recipients, but even with this large sample size, this did not reach statistical significance (Figure 2). We speculate that the strong immunosuppressants and/or the high incidence and severity of GVHD in HLA-mismatched related or unrelated HCT might somewhat diminish the protection from persisting host T cell immunity in NM-HCT. We confirmed that the onset of CMV disease was delayed, which resulted in a trend toward less CMV disease before day 100 in NM-HCT recipients (Table 2). Collectively, these data suggest that the residual CMV specific host memory cells had a limited or no effect on reactivation of CMV, but contributed to preventing progression to higher levels of viral load, at least early after less intensive conditioning. This is consistent with laboratory studies that showed a persistence of host memory T cells in HLA-matched related NM-HCT recipients [11,12]. However, after complete donor chimerism has been achieved in NM-HCT recipients, the benefits of protection against CMV infection seem to disappear. Our previous studies of CMV immunity showed that, after day 100, there was no difference in CMV-specific T cell immunity between NM-HCT and M-HCT recipients [11,12].

Somewhat surprisingly, more patients developed late CMV disease following NM-HCT compared to M-HCT. Further analysis suggested that the effect was driven by the earlier years of the study period [10] (Figure 3c). This was likely because of less virologic surveillance for late CMV infection and less use of late preemptive therapy, possibly because of the perception that infectious complications were less frequent and/or severe [10]. Additionally, the prolonged MMF prophylaxis or treatment, particularly in the unrelated NM-HCT, possibly contributed to more frequent incidence of late CMV disease in NM-HCT than in M-HCT. In more recent years, there was no increased risk of late CMV disease in NM-HCT recipients (Figure 3d). Also, the overall incidence of late CMV disease declined in both NM and M-HCT recipients, likely because of extended monitoring of CMV by PCR and increased use of preemptive anti-CMV treatment beyond day 100.

Risk factors for late CMV disease seen in this study (Table 4) were consistent with earlier reports by our group and others [19-21].

Although we were unable to separate the effect of GVHD on the risk of CMV endpoints in our previous studies [10,13], in the current study, both aGVHD and cGVHD were statistically significant risk factors for early and late CMV disease in the current study. This was consistent with previous reports [20].

The strengths of this study were the large sample size permitting multivariate modeling, well-defined and homogenous transplant protocols, highly standardized supportive care, CMV surveillance, and a comprehensive and standardized workup of bronchoalveolar lavage (BAL) and biopsy specimens (including autopsy specimens). Limitations were the retrospective nature of the analysis and that comedications could only be analyzed by protocol (as supposed to on a per-patient basis). Also, the data might not extend to different reduced-intensity protocols. Furthermore, in this study, the majority of the NM-HCT patients received peripheral blood stem cells (PBSCs) (Table 1). Although we could not detect a significant effect of the stem cell on CMV outcomes in the multivariate models, NM-HCT and PBSC use are tightly linked so we cannot conclusively rule out an interaction between the 2 modalities [22].

In conclusion, this large study provided robust data on the risk of CMV infection and disease in recipients of NM-HCT. The study confirmed and added statistical strength to some of the earlier findings, including the delayed onset of CMV disease and a lower risk of progression to higher viral loads during the first 100 days. Of note, the earlier reported trend toward an improved outcome of CMV disease after NM-HCT could not be confirmed in this study. In addition, the study showed that the survival rate after CMV disease was still very unfavorable, and emphasizes the need for improved prevention and treatment strategies for CMV. Maribavir, a novel antiviral agent [23] and immune enhancement strategies with CMV-specific T cells [24] or CMV vaccination [25] or preemptive strategies that combine virologic and immunologic monitoring may be options in this regard.

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